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Ubiquitin System: JAMMing in the Name of the Lid

Dispatch

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The isopeptide bonds formed by ubiquitin or its relatives are cleaved by hydrolases with active site cysteines. Recent studies have revealed that similar metalloprotease motifs — JAMMs — in the Rpn11 subunit of the 26S proteasome lid and in the Csn5 subunit of the COP9 signalosome are involved in deubiquitination and deneddylation, respectively.

The 26S proteasome is part of the most important protein-degrading system in eukaryotic cells, the ubiquitin/26S proteasome system, which is responsible for the specific and timely removal of regulatory proteins involved in essential cellular functions [1]. The 26S proteasome has two major parts: the 20S proteasome, which is the proteolytic core of the enzyme, and the 19S regulatory complex, which can in turn be dissected into two subcomplexes, the base and the lid [2]. Substrate proteins that carry a polyubiquitin signal are recognized by the proteasome, so that the substrate specificity of the system is determined at the level of the ubiquitinating enzymes — E1, E2 and, particularly, E3 — which act together to tag target proteins for destruction. Once bound to the 26S proteasome — possibly via the proteasome subunit Rpt2 [3] — polyubiquitinated proteins are unfolded by the base [4] and quickly degraded inside the 20S core.

How the lid of the proteasome contributes to the degradation process has beens an enigma for a long time. But now two independent studies [5,6] have shown that the lid component Rpn11/S13 is a metallo-deubiquitinase which removes polyubiquitin chains from substrates, a prerequisite for their further processing. Remarkably, these studies support data reported nine years ago by Hershko and co-workers [7] on an intrinsic ATP-dependent 26S proteasome deubiquitinase.

The proteasome lid is a highly conserved complex related to the COP9 signalosome and, to some extent, to the eukaryotic initiation factor 3 (eIF3) complex [2,8,9]. Rpn11 is the most conserved lid component and is homologous to the COP9 signalosome subunit Csn5. One might therefore expect that Csn5 would have metallo-deubiquitinase activity just like Rpn11. Surprisingly, as shown by a recent paper from Deshaies' group [10], in the context of the COP9 signalosome, Csn5 does not remove ubiquitin, but rather it removes the ubiquitin-like protein Nedd8 from appropriately modified – 'neddylated' — proteins.

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Back in 1993, it was shown by Hershko's group [7] that the 26S proteasome has ATP-dependent ubiquitin hydrolase activity. This activity is blocked by the heavy metal chelator o-phenanthroline but not by ubiquitin aldehyde, an inhibitor of ubiquitin carboxy-terminal hydrolases that have an active-site cysteine. Now, Verma *et al.* [5] suggest that a motif within the MPN (Mpr1, Pad1 N-terminal) domain of the Rpn11 proteasome subunit is responsible for this deubiquitinating activity. The MPN domain is found in two subunits of the proteasome lid complex, as well as in related proteins of the COP9 signalosome and the eIF3 complex. Interestingly some eukaryotic and all prokaryotic MPN domains have a highly conserved pattern of four charged amino acids — a glutamate residue followed by two histidines and an aspartate. This motif, $EX_nHXHX_{10}D$, has been dubbed JAMM, from Jab1/Pad1/MPN domain metallo-enzyme [5] (or MPN+ [11]). Verma *et al.* [5] propose that the histidine residues, in concert with the aspartate, bind a zinc ion which, together with the preceding glutamate, forms the catalytic site.

In order to test this hypothesis, the wild-type gene for Rpn11 in the budding yeast *Saccharomyces cerevisiae* was replaced by a version, *rpn11AXA*, encoding a mutant form of the protein in which the two critical histidines were substituted by alanine. This AXA mutation is lethal, and a temperature-sensitive yeast strain expressing a truncated form Rpn11 could be rescued by wild-type *rpn11* but not by *rpn11AXA*. Although these effects could be caused by the loss of proteasomal deubiquitination activity, they could also be due to various other defects resulting from the AXA mutation. To test this further, Verma *et al.* [5] isolated 26S proteasomes carrying the AXA mutation for *in vitro* deubiquitination and degradation experiments using the ubiquitinated cyclin-dependent kinase (Cdk) inhibitor Sic1 as a substrate. Although the 26S proteasome containing the AXA mutant form of Rpn11 seemed to have a relatively normal gross structure, the authors found it is indeed deficient in deubiquitination and degradation activity [5].

Similar conclusions have been reached by Yao and Cohen [6] in their independent study of the putative metalloprotease activity of Rpn11. These authors substituted the conserved histidine and glutamate residues in Rpn11's JAMM motif, again finding the mutations are lethal to budding yeast at 30°C. Maytal-Kivity *et al.* [11] found the histidine mutation is not lethal, but causes thermosensitivity with slow growth at 25°C. Yao and Cohen [6] also performed experiments with classical ubiquitin hydrolase inhibitors, such as ubiquitin aldehyde and ubiquitin vinylsulfone, or metal chelators that block metalloproteases, and their results support the idea that the intrinsic deubiquitination activity of the 26S proteasome, described nine years ago [7], can most likely be assigned to the JAMM motif in Rpn11's MPN domain.

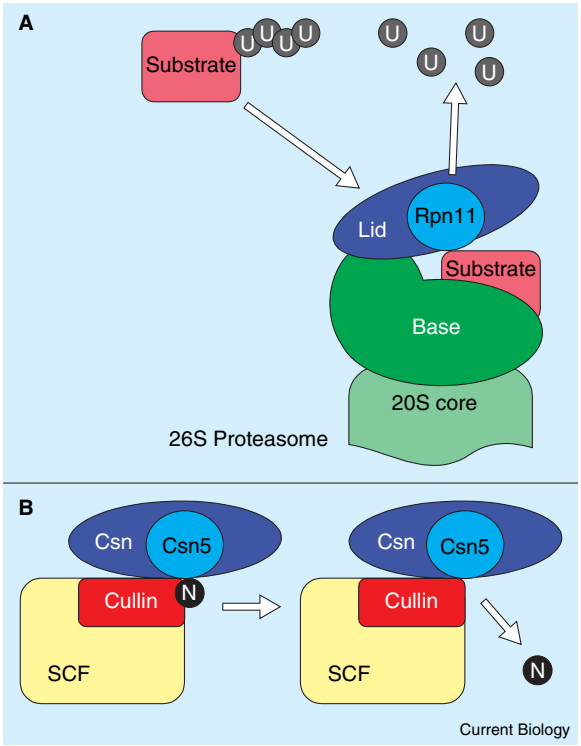

Figure 1. A model for the roles of the JAMM-containing proteins Rpn11 and Csn5.

(A) For degradation by the 26S proteasome, substrates have to be deubiquitinated upon binding to the protease complex. This process involves the lid component Rpn11 in a JAMM-dependent manner. (U, ubiquitin.) (B) The cullin component of the SCF complex is deneddylated by the COP9 signalosome with the Csn5 subunit, again depending on its JAMM motif. (N, NEDD8; CSN, COP9 signalosome.)

Interestingly, this deubiquitination activity is ATP-dependent. Verma *et al.* [5] were only able to detect the JAMM-dependent deubiquitination activity with fully assembled 26S proteasome, and not with the 19S regulator or its Rpn11-containing lid subcomplex [5]. Yao and Cohen [6], however, found that the 19S regulator on its own can cleave ubiquitin conjugates in an ATP-independent manner. The different results might reflect different substrates used by the two groups, though both found that neither recombinant Rpn11 nor the lid complex alone have deubiquitination activity [5,6]. So it is conceivable that other proteasomal subunits are required to assist Rpn11 in the deubiquitination process, perhaps by positioning the substrate properly within the proteasome. In a model presented by Verma *et al.* [5], the multiubiquitinated substrate binds to the proteasome, where it is unfolded and threaded into the 20S core while the ubiquitin chain is cleaved off by Rpn11. When deubiquitination is deficient, the bulky multiubiquitin moiety might sterically block further translocation of the polypeptide chain into the 20S core [5].

Remarkably, the JAMM of Csn5 promotes cleavage, not of ubiquitin, but rather of the related modifier Nedd8. Nedd8 is one of a group of ubiquitin-like proteins, and is more similar to its famous cousin than all other members of the family [12]. In a similar manner to ubiquitin, Nedd 8 is activated by E1 and E2 enzymes and eventually conjugated to target proteins. So far, the only known targets of neddylation are the cullins. Cullins are core components of a special class of E3 ubiquitin ligases, the best characterised of which is the SCF complex of Skp1, a variable F-box protein, cullin 1 and Roc1 [13]. SCF complexes mark a number of important regulators of cell proliferation for destruction, among them p27, E2F and β catenin. The significance of cullin neddylation is poorly understood, but it has been shown that Nedd8 helps to recruit the E2 enzyme to SCF, thereby stimulating its ubiquitin ligase activity [14].

Last year, the COP9 signalosome was identified as a novel interacting partner of SCF complexes which promotes cullin deneddylation [15]. The nature of the deneddylating activity has since proved to be enigmatic. Deneddylation can be inhibited by the alkylating agent NEM, an inhibitor of cysteine proteases, casting suspicion on the subunit Csn5, which has a cysteine protease motif [16]. But the assumption that Csn5 really is the Nedd8 isopeptidase came into doubt when mutation of its putative active site cysteine failed to eliminate deneddylation [16].

Now, Cope *et al.* [10] have pinpointed Csn5's MPN domain as the site of the metalloprotease activity that cleaves Nedd8 from cullins. Deneddylation by the COP9 signalosome is inhibited by the metal chelators EDTA and o-phenanthroline, and reactivated by nickel ions.

Deletion of the gene for *Csn5* in the fission yeast *Schizosaccaromyces pombe* results in Cul1 being present only in its neddylated state. Deneddylation activity is restored upon ectopic expression of wild-type *Csn5*, but not of a version mutated in the conserved histidine and aspartate residues of the JAMM motif. The physiological importance of an intact JAMM domain in Csn5 is illustrated by the deficiencies in photoreceptor neuron differentiation and reduced viability caused by JAMM mutations of Csn5 in the fruit fly *Drosophila*.

As predicted, then, JAMMs form parts of metalloprotease active sites which, in the case of Rpn11 and the 26S proteasome can deubiquitinate, and in the case of Csn5 and the COP9 signalosome can deneddylate their targets. It will be interesting to see whether prokaryotic relatives of Rpn11 and Csn5 also have metalloprotease activity. One might also ask why the COP9 signalosome appears to be sufficient for Csn5-mediated deneddylation, whereas the proteaseome lid complex is not sufficient for Rpn11-mediated deubiquitination. Are there additional COP9 signalosome-associated factors that support Csn5 activity? Do COP9 signalosome-associated kinases [17] have an effect on deneddylation? And is the ATP dependency of Rpn11-mediated deubiquitination solely due to the ATP requirement for 26S proteasome integrity?

Interestingly, Rpn11-catalysed deubiquitination is essential for viability in budding yeast, whereas Csn5-mediated deneddylation is not. Deubiquitination is a prerequisite for protein degradation, while deneddylation stabilizes substrates (by reducing SCF ubiquitin ligase activity). Is there specific regulation that

coordinates deneddylation and deubiquitination? Is deneddylation the only activity of the COP9 signalosome that regulates SCF-mediated ubiquitination?

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